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14. ABSTRACT

Phytochemical investigation of the aerial parts of Zygophyllum coccineum L. led to the isolation of nine ursane-type triterpene saponins (1?9), including the new one; zygophylloside S (1), together with a known flavonoid glycoside (10) and a sterol glycoside (11).The isolated compounds were tested for antifungal activity against several important plant pathogens and for insecticidal activity against two important mosquito species. Among the isolated compounds 1, 3, 5, 6, and 9 showed 32?77% fungal growth inhibition at a concentration of 30 μM against Phomopsis viticola. Compound 9 showed 90% and 80% mosquitocidal activity at 3.1 μg/0.5μL against Aedes aegypti and Culex quinquefasciatus, respectively.

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Triterpenoidal Saponins: Bioactive Secondary Metabolites from *Zygophyllum coccineum*

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Abstract

Phytochemical investigation of the aerial parts of *Zygophyllum coccineum* L. led to the isolation of nine ursane-type triterpene saponins (**1**–**9**), including the new one; zygophylloside S (**1**), together with a known flavonoid glycoside (**10**) and a sterol glycoside (**11**).The isolated compounds were tested for antifungal activity against several important plant pathogens and for insecticidal activity against two important mosquito species. Among the isolated compounds **1, 3, 5, 6**, and **9** showed 32–77% fungal growth inhibition at a concentration of 30 µM against *Phomopsis viticola*. Compound **9** showed 90% and 80% mosquitocidal activity at 3.1 µg/0.5 µL against *Aedes aegypti* and *Culex quinquefasciatus*, respectively.

Key words
Zygophyllum coccineum L. · Zygophyllaceae · ursane-type triterpene glycosides · zygophylloside S · antifungal activity · insecticidal activity

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Saponins are widely distributed in the plant kingdom and have a wide range of biological properties. Several investigations have reported the anti-inflammatory, antibacterial, antifungal, antiparasitic, antiviral, hemolytic, and cytotoxic activities of saponins [1]. As a part of a research program aimed at identifying new natural fungicides and insecticides, *Z. coccineum* L., a saponin-rich plant, was chosen for further investigation.

Z. coccineum L., Zygophyllaceae, grows wild in Egyptian deserts [2]. It has been used in traditional medicine as antihelminthic, diuretic, antidiabetic, antiasthma, antigout, antirheumatic, and antihypertension agents [3–5].

Zygophylloside S (**1**) was isolated as a white powder from the *n*-butanol-soluble part of the methanol extract by repeated flash and gravity column chromatography over normal and reversed phase (RP-18) silica gel. The HR-ESI-MS exhibited an $[M + Na]^+$ ion at $m/z = 803.4166$ (calcd. 803.4097) that is consistent with the molecular formula $C_{41}H_{64}O_{14}$. The ^{1}H-NMR spectrum of **1** displayed resonances for four tertiary methyls [$\delta_H = 0.88$ (s), 1.06 (s), 1.10 (s), 1.14 (s)], two secondary methyls [($\delta_H = 0.82$ (d, $J = 6.4$ Hz) and 1.23 (d, $J = 6.0$ Hz)], an olefin proton [$\delta_H = 6.01$ (s)], and an oxygenated methine [$\delta_H = 3.15$ (dd, $J = 12.0$, 4.0 Hz)]. The ^{13}C-NMR spectrum showed 41 resonances, including characteristic ones at $\delta_C = 180.4$ and 178.3 due to two carboxy groups and at $\delta_C = 134.4$ and 129.3 due to a double bond. The DEPT-135 experiment allowed differentiation of the 41 carbon resonances into six methyl, 11 methylene, 16 methine, and 8 quaternary carbons, of which 6 methyl, 9 methylene, 7 methine, and 8 quaternary carbons were attributed to the aglycon moiety. This data indicated an ursane-type triterpene skeleton for the aglycon, with a classical olefin bond at C-12, and two carboxy groups at C-27 and C-28, corresponding to quinovic acid [6]. In addition, the ^{1}H- and ^{13}C-NMR spectra showed resonances for two sugar units, assignable to *β*-glucopyranose [$\delta_H = 4.76$ (d, $J = 7.6$ Hz, H-1′); $\delta_C = 105.3$ (C-1′), 84.2 (C-2′), 78.5 (C-3′), 71.9 (C-4′), 78.2 (C-5′) and 63.1 (C-6′)] and *α*-arabinopyranose [$\delta_H = 5.14$ (d, $J = 6.8$ Hz, H-1″); $\delta_C = 106.9$ (C-1″), 74.0 (C-2″), 74.6 (C-3″), 69.5 (C-4″) and 67.3 (C-5″)]. The long-range HMBCs observed from H-1′ to C-3 and from H-1″ to C-2′ revealed that the glucose was linked at C-3 and the arabinose at C-2′. The aglycone was recognized from the aforementioned ^{1}H- and ^{13}C-NMR data (**Table 1**) as 3*β*-hydroxyurs-12-ene-27,28-dioic acid. The assignment of the ^{1}H- and ^{13}C-NMR data was facilitated by comparison with those of *Zygophyllum* saponins [6–11] and confirmed by HMQC, HMBC (**Fig. 1**), and COSY spectra. Accordingly, zygophylloside S (**1**) was elucidated as 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-glucopyranosyl] quinovic acid [12].

By comparing their spectral and physical data with those of known compounds reported in the literature [6–11,13], compounds **2**–**11** were characterized as 3-*O*-[*β*-D-(2-*O*-sulphonyl)-quinovopyranosyl] quinovic acid (**2**), 3-*O*-[*β*-D-glucopyranosyl] quinovic acid (**3**), 3-*O*-[*β*-D-glucopyranosyl] quinovic acid-28-*O*-*β*-D-glucopyranosyl ester (**4**), 3-*O*-[*β*-D-quinovopyranosyl] quinovic acid-28-*O*-*β*-D-glucopyranosyl ester (**5**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-quinovopyranosyl] quinovic acid (**6**), 3-*O*-[*α*-L-arabinopyranosyl-(1 → 2)-*β*-D-quinovopyranosyl] quinovic acid-28-*O*-*β*-D-glucopyranosyl ester (**7**), 3-*O*-[*β*-D-(2-*O*-sulphonyl)quinovopyranosyl] quinovic acid-28-*O*-*β*-D-glucopyranosyl ester (**8**), and 3-*O*-[*β*-D-(2-*O*-sulphonyl)glucopyranosyl] quinovic acid (**9**), isorhamnetin-3-*O*-rutinoside (**10**), and *β*-sitosterolglucoside (**11**).

The antifungal activity of the compounds was investigated against *Colletotrichum acutatum, C. fragariae, C. gloeosporioides, Botrytis cinerea, Phomopsis obscurans, P. viticola,* and *Fusarium oxysporum* and was observed against *P. viticola* after 144 h exposure. Among the isolated compounds, compound **6** showed 76.9% ± 3.7% fungal growth inhibition at 30 µM, followed by compounds **1** (55.6% ± 4.0%), **5** (54.1% ± 7.7%), **3** (44.3% ± 6.2%), and **9** (31.5% ± 7.0%) (**Fig. 2**), while captan used as the standard showed growth inhibition of 99.9% ± 0.3%.

Isolated compounds were subjected to a high-throughput larval bioassay with *Aedes aegypti* and evaluated for adult toxicity against *A. aegypti* and *Culex quinquefasciatus*. Although no mortality was observed with any of the compounds against first instar larvae of *A. aegypti*, compound **9** exhibited significant adult mortality of 90% and 80% at 3.1 µg/0.5 µL concentration against *A. aegypti* and *C. quinquefasciatus*, respectively. This is the first report of the antifungal and insecticidal activity of compounds **1, 3, 5, 6**, and **9**.

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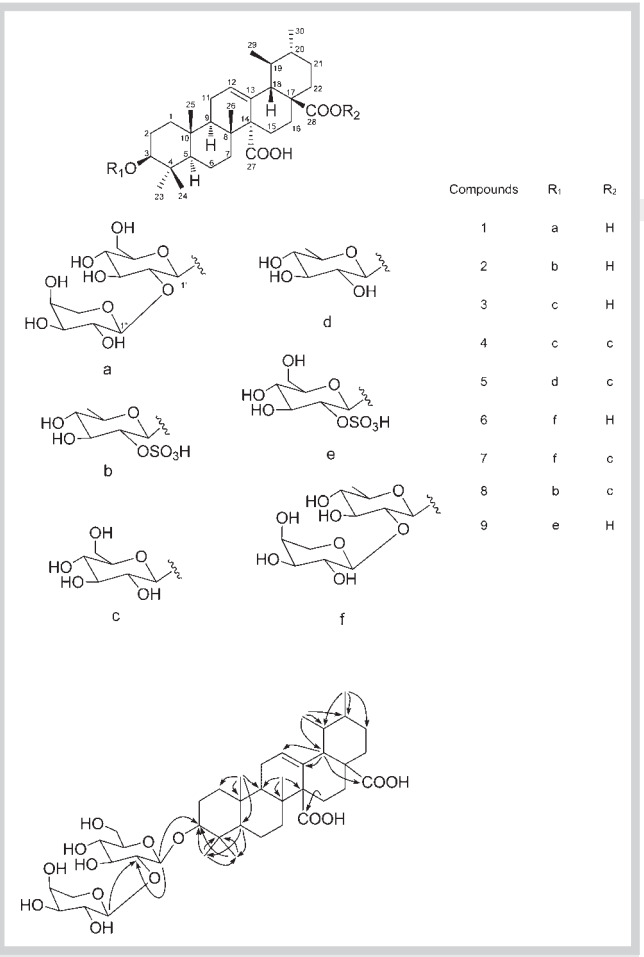

Fig. 1 HMBCs of zygophylloside S (**1**).

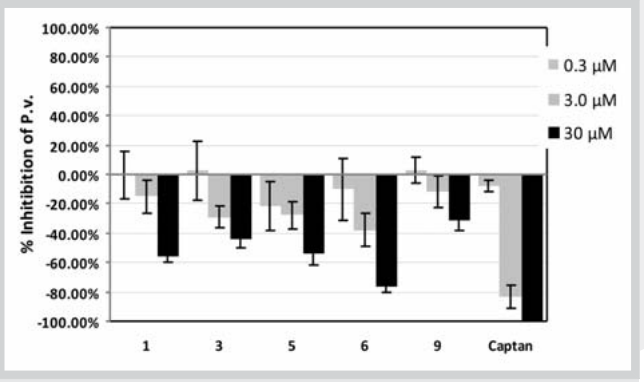

Fig. 2 Growth inhibition of compounds **1, 3, 5, 6**, and **9** against *Phomopsis viticola* (P.v.) using a 96-well microdilution broth assay.

Materials and Methods

The aerial parts of *Z. coccineum* L. were collected from the eastern desert in Egypt in May 2008 and identified by Dr. M. Elgebaly, Prof. of Taxonomy, Faculty of Science, Cairo University. A voucher specimen has been deposited at the Herbarium of Faculty of Science, Beni Suef University.

Table 1 ^{1}H- and ^{13}C-NMR data for zygophylloside S (**1**) in pyridine-d_5.

Position	δ_C	δ_H[a]	Position	δ_C	δ_H[a]
1	39.4	1.51, 1.02	22	37.8	1.40, 1.69
2	27.1	1.86, 1.95	23	28.0	1.14 s
3	89.0	3.15 dd (12.0, 4.0)	24	16.9	1.06 s
4	39.8	–	25	16.8	0.88 s
5	56.1	0.90	26	19.2	1.10 s
6	18.9	1.51, 1.31	27	178.3	–
7	37.4	1.98, 1.86	28	180.4	–
8	40.3	–	29	18.6	1.23 d (6.0)
9	47.4	2.67 dd (11.2, 5.0)	30	21.7	0.82 d (6.4)
10	37.3	–	1′	105.3	4.76 d (7.6)
11	23.6	1.90, 2.12	2′	84.2	4.06 t like (8.6)
12	129.3	6.01 s	3′	78.5	4.25 t like (8.6)
13	134.4	–	4′	71.9	4.20 t like (8.6)
14	57.1	–	5′	78.2	3.87 m
15	25.8	2.35, 2.61	6′	63.1	4.36, 4.52 dd (15.2, 3.6)
16	26.7	2.23, 2.24	1′′	106.9	5.14 d (6.8)
17	49.0	–	2′′	74.0	4.54 t like (8.0)
18	55.2	2.80 d (11.3)	3′′	74.6	4.17
19	38.1	1.40	4′′	69.5	4.30
20	39.7	0.80	5′′	67.3	3.75 d (11.3), 4.36
21	30.9	1.30, 1.40			

Note: δ values are in ppm; J values (in Hz) are in parentheses. [a] Multiplicity is not clear for some signals due to overlapping

The dried, powdered aerial parts of *Z. coccineum* (2 kg) were extracted with 80% methanol (10 × 4 L) and evaporated under reduced pressure to give a residue (300 g). A part (150 g) was dissolved in water and sequentially fractionated with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The *n*-butanol extract (20 g) was chromatographed over a silica gel column (600 g, 8 × 60 cm) by using EtOAc/$CHCl_3$/MeOH/H_2O 15 : 8 : 4 : 1 (10 L) and 6 : 4 : 4 : 1 (6.5 L) to yield seven main fractions (A–G). Fraction D (300 mg) was chromatographed on a Biotage system [SP-1 (40 + M), C18 column, MeOH/H_2O 3 : 2 (2 L)] followed by silica gel CC [20 g, 1 × 35 cm, $CHCl_3$/MeOH/H_2O 4 : 1 : 0.1 (150 mL)] to afford **1** (9 mg).

Zygophylloside S (**1**): White powder; $[\alpha]_D^{20}$: +0.050 (*c* 0.01, MeOH); IR (NaCl): ν_{max} = 3368 (OH), 1687, 1224 (CO) cm^{-1}; HR-ESI-MS (positive-ion mode): *m*/*z* = 803.4166; ^{1}H- and ^{13}C-NMR spectroscopic data: see **Table 1**.

Determination of sugars of 1

Compound **1** (3 mg) was heated with 2 N HCl at 95 °C for 3 h. The mixture was then neutralized with NH_4OH and extracted with EtOAc (2 × 2 mL). The aqueous layer residue was dissolved in pyridine and 0.1 M cysteine methyl ester hydrochloride in pyridine was added. The reaction mixture was heated at 60 °C for 1 h. An equal volume of phenyl isothiocynate in pyridine was added and heated at 60 °C for 1 h. The mixture was filtered and analyzed by reversed-phase HPLC [Waters Alliance 2695, equipped with photodiode array detector, and Luna C18 column (150 × 4.6 mm, 5 µm particle size; Phenomenex, Inc.)], using acetonitrile + 0.1% acetic acid (A) and water + 0.1% acetic acid (B) in a gradient mode: A/B 10/90 for 20 min and A/B 55/45 for the next 25 min, at a rate of 1 mL/min. The response was detected at 250 nm. The standard sugar derivatives were prepared and analyzed identically. L-Arabinose and D-glucose were identified by comparison of the reten-

tion times of their derivatives with those of authentic sugar samples [L-arabinose: 13.4 min (minor)/15.5 min (major); D-arabinose: 13.3 min (minor)/15.7 min (major); D-glucose: 12.3 min (minor)/15.0 min (major); L-glucose: 12.8 min (minor)/14.9 min (major)].

Antifungal assay

Isolated compounds were evaluated using a 96-well microbioassay system for antifungal activity against *Colletotrichum acutatum, C. fragariae, C. gloeosporioides, Botrytis cinerea, Phomopsis obscurans, P. viticola*, and *Fusarium oxysporum* [14]. The technical-grade commercial fungicide captan (98%, Chem Service, Inc.) was used as a standard.

Mosquitocidal assay

Isolated compounds were subjected to a high-throughput larval bioassay with *Aedes aegypti* and evaluated for adult toxicity against *A. aegypti* and *Culex quinquefasciatus* [15, 16]. Permethrin (55% *cis* and 45% *trans*; Chem Service, Inc.) was used as a standard.

Supporting information

Detailed protocols for biological assays, general experimental procedures, thorough extraction/isolation procedures, and NMR spectra for compound **1** are available as Supporting Information.

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